Local delivery of an antisense oligonucleotide for recessive dystrophic epidermolysis bullosa

R Mendes, L van Wissen, MW de Jager, F van der Ham, V Brinks, T Ritsema, EM Haisma | ProQR Therapeutics N.V. Leiden, the Netherlands

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic blistering disorder caused by mutations in the COL7A1 gene encoding for type VII collagen (C7) protein. We have demonstrated that QR-313, a single stranded antisense oligonucleotide (AON), is efficient in the skipping of exon 73 from COL7A1 in human skin equivalents, thereby removing mutations in exon 73 of COL7A1 and enabling the restoration of C7 protein expression (figure 1)^{1,2}. One of the main hurdles in development of potential medicines based AONs is sufficient delivery to the target organ. It has been shown that systemic (intravenous) administration leads to high AON concentrations in the liver and kidneys, with hardly detectable delivery to the skin. Hydrogels are part of the daily care of RDEB patients, therefore a conventional carbomer based hydrogel was developed for QR-313.

Aims

In this study we wanted to assess Cy5-labeled QR-313 delivery in different (wounded) models, including human skin equivalents (HSEs), ex vivo human skin, ex vivo porcine skin and an in vivo wound model using minipigs. Cy5-labeled QR-313 formulated into PBS or hydrogel was applied.

Figure 1. Mode of action of QR-313. Prevention of exon 73 inclusion in the mRNA of COL7A1 to create a slightly shorter C7 protein.

Material and Methods

Full thickness HSEs were grown from healthy skin donors in an air-liquid interface on filters. Ex vivo human skin was obtained, washed and excess dermis was removed manually, next the skin was prepped to fit onto filters to culture at the air-liquid interface. Ex vivo porcine skin was obtained, the skin was prepped to 1 mm thickness using a dermatome and cultured at the air liquid interface. An *in vivo* minipig model was used, where 0.35 mm wounds were created using a dermatome. In all models a DEB-like wound was created by removing the epidermis, either with a scalpel or with a dermatome. Next Cy5-labeled QR-313 was applied onto the skin pieces in PBS or formulated in a hydrogel. Cy5 diffusion into the skin models was followed for 24 hrs up to 7 days. All skin sections were processed for histology, stained with DAPI or haematoxylin and analyzed using (fluorescent) microscopy.

Results

1. Cy5-labeled QR-313 is delivered in wounded human skin equivalents

Human skin equivalents (HSEs) were used to assess behavior of the AON in a 3D environment. Part of the epidermis was removed to mimic DEB wounds before application of Cy5-labeled QR-313 formulated into PBS (0.5 mg/ml) or hydrogel (0.5 mg/g). After 24 hours no penetration into the epidermis of intact models was observed. In the HSEs where part of the epidermis was removed diffusion into the dermal compartment of the model was observed. When Cy5-labeled QR-313 was formulated into the hydrogel more diffusion also lateral underneath the epidermis was observed (figure 2).

Figure 2. Delivery in human skin equivalents of Cy5-labeled QR-313 into two different formulations after 24 hrs. Top row shows application of Cy5-labeled QR-313 formulated in PBS, bottom row formulated in hydrogel. Cross sections of FFPE skin equivalents were counterstained with DAPI (blue) and the Cy5 QR-313 signal (red) was visualized by fluorescence microscopy.

Results (continued)

Next delivery of different concentrations of QR-313 in hydrogel were assessed (10, 100 or 1000µg per HSE). Diffusion of Cy5 QR-313 into the dermis underneath the intact epidermis was seen at all concentrations. The signal intensified with increasing concentrations of QR-313 in both the wound bed and underneath the epidermal layer. This suggests more diffusion at higher QR-313 concentrations; in a dose-proportional manner (figure 3).

Figure 3. Delivery of different concentrations Cy5-labeled QR-313 formulated in hydrogel in HSEs. Three different concentrations were tested: 10, 100 or 1000µg Cy5-labeled QR-313 was applied per HSE for 24 hrs. Cross sections of FFPE sections were counterstained with DAPI and the Cy5 signal was visualized by fluorescence microscopy.

2. Cy5-labeled QR-313 is delivered in the dermis of wounded ex vivo porcine and human skin

Application of Cy5-labeled QR-313 for 24 or 48 hrs onto intact and wounded ex vivo porcine skin demonstrated diffusion of Cy5-labeled QR-313 into the dermis, whereas in intact skin no Cy5-labeled QR-313 was taken up. QR-313 formulated in hydrogel showed a deeper penetration of the dermis than QR-313 in solution (PBS) (figure 4). Similar results were obtained after application of Cy5-labeled QR-313 formulated into hydrogel or PBS onto ex vivo human skin for 24 hrs. Diffusion of the oligo was visible in skin where the epidermis was removed, almost no lateral diffusion was observed at the wound edges (figure 5).

Figure 4. QR-313 delivery in ex vivo porcine skin of Cy5-labeled QR-313 formulated in either hydrogel (left column) or PBS (right column) 24 hrs and 48 hrs after application. Cy5-labeled QR-313 is indicated in red. Scale bar indicates 100µm.

Figure 5. Delivery of Cy5-labeled QR-313 formulated into hydrogel on ex vivo human skin after 24 hrs. Cross sections of FFPE skin were counterstained with DAPI and the Cy5 QR-313 signal was visualized by fluorescence microscopy. Scale bar indicates 200µm.

3. Cy5-labeled QR-313 is delivered in nuclei after application on wounded minipig skin

Wounds of 2x3 cm and 0.35 mm deep were created onto the backs of minipigs. Delivery to the dermis was observed using Cy5 QR-313 at a strength of 0.5 mg/g (50mg/cm²) in hydrogel after 3 times dosing using a 48 hour dose interval. After 7 days the wounds were closed by a newly formed epidermis and the Cy5 signal was seen in the upper dermis and throughout the newly formed epidermal cell layers. Confocal microscopy shows co-localization of the Cy5 label with the cell nuclei (figure 6). In agreement with the experiment with ex vivo models, no penetration of Cy5-labeled QR-313 was observed into intact skin.

Figure 6. Cy5-labeled QR-313 delivery to wounded skin of minipigs. Cy5-labeled QR-313 was applied 3 times with 48 hrs intervals, 7 days after wounding biopsies were taken and cross sections of FFPE skin were counterstained with Hoechst (blue) and the Cy5 signal (red) was visualized by confocal microscopy.

Discussion

QR-313 is designed to exclude exon 73 from COL7A1 (see poster: In vitro evaluation of QR-313; an anti-sense oligonucleotide designed to skip exon 73 from the COL7A1 mRNA). In order to perform its function, QR-313 should be present in the nucleus. Based on the confocal images of the *in vivo* delivery in minipig it is reasonable to assume that QR-313 is efficiently delivered to the cell nucleus *in vivo*.

Conclusion

Here it is demonstrated that Cy5-labeled QR-313 delivery in vitro, ex vivo and in vivo models is successful in wounded skin, but not in intact skin. We conclude that hydrogel is a suitable formulation that enables diffusion of QR-313 into the skin of DEB patients at wound sites.

Literature

1. Turczynski, S., Titeux, M., Tonasso, L., Décha, A., Ishida-Yamamoto, A., & Hovnanian, A. (2016). Targeted Exon Skipping Restores Type VII Collagen Expression and Anchoring Fibril Formation in an In Vivo RDEB Model. *Journal of Investigative Dermatology*, 136(12), 2387–2395. <http://doi.org/10.1016/j.jid.2016.07.029>
2. Bornert et al., 2016. Functional evaluation of targeted exon deletion reveals prospect for dystrophic epidermolysis bullosa therapy. *Molecular Therapy* accepted article preview online 09 May 2016; doi:10.1038/mt.2016.92

Scan the code for a digital copy

<http://www.proqr.com/deb20se17d/>